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Lactobacillus rhamnosus Meningitis following Recurrent Episodes of Bacteremia in a Child Undergoing Allogeneic Hematopoietic Stem Cell Transplantation▽

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We report a case of meningitis due to *Lactobacillus rhamnosus* in a child undergoing allogeneic hematopoietic stem cell transplantation for acute leukemia. Four episodes of bacteremia involving strains with pulsotypes identical to that of the cerebrospinal fluid isolate preceded meningitis. After several courses of clindamycin, no relapse occurred during the patient follow-up.

CASE REPORT

In December 2005, a 10-year-old boy was admitted to the department of pediatric hematology in the Teaching Hospital of Clermont-Ferrand (France) for allogeneic cord blood transplantation. He was suffering from Philadelphia chromosome-positive preB2 acute lymphoblastic leukemia. He had been treated in accordance with the induction phase of the European Intergroup Study on Post-Induction Treatment of Ph^+ ALL protocol but had not responded. He finally received a partially matched unrelated cord blood transplant (mismatch 4/6) after myeloablative conditioning consisting of total body irradiation (12 Gy) and administration of cyclophosphamide at 120 mg/kg on 13 March 2006. After the graft, he was treated with cyclosporine and corticosteroids to prevent graft-versus-host disease until day 36 and with acyclovir (4.5 g once a day [q.d.]), sulfamethoxazole (1 g 3 times a week) and gamma globulins (16 g once a week) to prevent infectious complications. He was in complete aplasia until day 26 posttransplantation. He continued to suffer from neutropenia until day 85 and from thrombocytopenia until his discharge despite a late but successful engraftment. His polymorphonuclear neutrophil count stayed below 1×10^9 cells/liter throughout the hospital stay.

On day 29, although being empirically treated with ceftazidime (6 g q.d.), amikacin (600 mg q.d.), and vancomycin (1.2 g q.d.) for a first febrile episode that had occurred on day 6, he developed a fever (40.3°C) with clinical sepsis and a C-reactive protein (CRP) level of 240 mg/liter. Fifteen sets of aerobic and anaerobic blood cultures (Becton Dickinson Diagnostic Instrument Systems) were performed between day 29 and day 33 of which 7 became positive with a Gram-positive rod after 3 days of culture on Columbia agar with 5% sheep blood (Oxoid, Dardilly, France). This bacterium was nonmotile, non-spore forming, and facultatively aerobic. It was catalase negative and oxidase negative. The Rapid ID 32A kit (bioMérieux, LaBalme, France) identified the bacterium as *Lactobacillus acidophilus*, while the API 50 CH test kit and API CHL medium (bioMérieux) identified it as *L. rhamnosus*. 16S rRNA gene amplification and sequencing were performed as described previously, using primers 91E (5′-TCAAA[G,T]GAA TTGACGGGGGC-3′), 13BS (5′-GCCCGGGAACGTATTC AC-3′), F1 (5′-AGAGTTTGATCCTGGCTGAG-3′), F2 (5′-GTGCCAGCAGCCGCGG-3′), R1 (5′-TCTACGCATTCCA CCGCTAC-3′), and R2 (5′-GGGTTGCGCTCGTTG-3′) (12, 14). Comparison with sequences deposited in the GenBank database showed that the partial sequence obtained (1,392 bp, accession number GU386370) shared 99.9% identity with those of the 16S rRNA genes of *L. rhamnosus* strain Lcr35 (accession number EU184020) and *L. rhamnosus* ATCC 53103 (accession number AP011548).

Susceptibility testing was performed by the disk diffusion method (Mast Diagnostic, Amiens, France) on Mueller-Hinton agar with 5% sheep blood (Bio-Rad, Marnes-la-Coquette, France) and was interpreted according to the guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie related to streptococci as previously described (8, 11). The strain was susceptible to ampicillin, rifampin, erythromycin, lincomycin, and pristinamycin and was resistant to benzylpenicillin, cefotaxime, all aminoglycosides, trimethoprim-sulfamethoxazole, fluoroquinolones, fusidic acid, tetracycline, and vancomycin. The MICs of benzylpenicillin, vancomycin, and erythromycin were determined by Etest (AB Biodisk, Solna, Sweden) and interpreted in accordance with the CLSI recommendations (7). The MIC results confirmed the resis-

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tance to vancomycin (>256 μg/ml) and benzylpenicillin (2 μg/ml) and the susceptibility to erythromycin (0.064 μg/ml). The child's treatment was switched on day 34 to amoxicillin (8 g q.d.) and clindamycin (1.8 g q.d.).

After 18 days of antibiotic treatment, clindamycin was stopped and amoxicillin was continued alone. On day 67, he had another febrile episode (40°C) with a CRP level of 168 mg/liter. Empirical treatment with amikacin (600 mg q.d.) and vancomycin was added to amoxicillin, but seven blood cultures became positive for *L. rhamnosus* with the same pattern of antimicrobial resistance as the previous isolate. Transesophageal echocardiography revealed no vegetation. Imipenem (2 g q.d.) and clindamycin (1.8 g q.d.) were added to the antibiotic treatment regimen until day 78. On day 86, a new blood culture became positive for *L. rhamnosus* despite treatment with amoxicillin. Clindamycin was reintroduced and continued until day 95.

On day 102, the patient developed a fever (39.2°C) with a CRP level of 144 mg/liter and a new blood culture was positive for *L. rhamnosus*. Clindamycin was added to amoxicillin and to empirical treatment with ceftazidime (6 g q.d.), amikacin (600 mg q.d.), and vancomycin (1.2 g q.d.). The fever decreased, and clindamycin was stopped on day 106, but on day 113, the patient had a headache with impaired consciousness and confusion suggestive of meningitis. Lumbar puncture showed 1 red cell and 4 leukocytes/mm^3, glucose at 4.3 mmol/liter (serum glucose, 4.8 mmol/liter), and proteins at 0.21 g/liter of cerebrospinal fluid (CSF). However, the CSF culture was positive for *L. rhamnosus*. Clindamycin was then reintroduced. All blood and CSF cultures were negative after day 113. Direct 16S rRNA PCR performed on a CSF sample on day 121 was negative. The patient was discharged on day 140. Amoxicillin and clindamycin were stopped on day 154.

In this patient, *L. rhamnosus* was isolated from 16 blood cultures sampled over a 73-day period and from a CSF sample. The molecular comparison by pulsed-field gel electrophoresis (PFGE) (9) of the strains isolated during the different infectious episodes, i.e., four successive episodes of bacteremia and meningitis, revealed that they displayed identical pulsotypes (Fig. 1). The origin of the infection remained unknown. The patient received no probiotic during his hospital stay. Moreover, the PFGE pattern clearly differed from those observed for both probiotics *L. rhamnosus* Lcr35 and *L. rhamnosus* ATCC 53103, so-called *L. rhamnosus* GG (9). Colonoscopy was performed on day 101 and revealed no anomaly that could have favored the translocation of the *Lactobacillus*. The patient had no further *Lactobacillus* infection but finally died, 1 year later, from an infection caused by *Pseudomonas aeruginosa*.

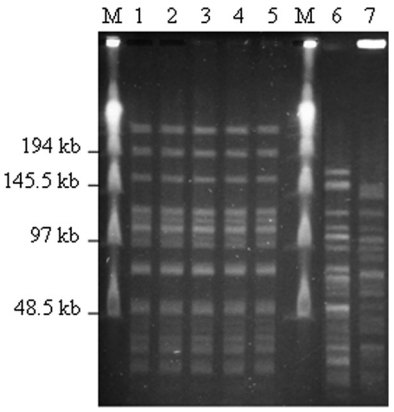

FIG. 1. Comparison of the PFGE patterns of NotI-restricted DNA from five *L. rhamnosus* strains isolated from the patient during successive infectious episodes with those of probiotic strains *L. rhamnosus* 35 and *L. rhamnosus* ATCC 53103. Lanes M, concatemer of phage lambda DNA used as a molecular weight ladder. *Lactobacillus* strains: lane 1, isolate from blood culture on day 29; lane 2, isolate from blood culture on day 67; lane 3, isolate from blood culture on day 86; lane 4, isolate from blood culture on day 102; lane 5, isolate from CSF on day 113; lane 6, *L. rhamnosus* strain Lcr35; lane 7, *L. rhamnosus* strain ATCC 53103 (strain GG).

Lactobacilli are considered commensal bacteria of the human oral, gastrointestinal, and genital tracts. Some species of *Lactobacillus* are used as probiotic bacteria and have been shown to be effective in the treatment of diverse infections, particularly diarrhea (2). However, these Gram-positive rods may be responsible for infections, mainly found in patients with compromised defenses and severe underlying diseases (6). They have been mainly implicated in bacteremia and endocarditis and more rarely in localized infections (6). These infections are sometimes associated with the use of *Lactobacillus* as a probiotic agent, especially in immunocompromised patients, but the risk is not clearly established (4, 13). Diagnosis of these infections can be problematic because phenotypic identification of the *Lactobacillus* species by most identification kits is unreliable and 16S rRNA gene sequencing is required (17). Indeed, recognizing these pathogens is crucial, their treatment requiring specific antimicrobial agents since several *Lactobacillus* species are resistant to drugs usually used in severe infections due to Gram-positive bacteria like glycopeptides (16).

In the present case, the patient had neutropenia until day 85 and his polymorphonuclear neutrophil count was below 1×10^9 cells/liter until he was discharged. Such an immunodepressive condition is clearly a risk factor for a *Lactobacillus* infection (15). Sequencing of a large part of the 16S rRNA gene allowed the accurate identification of the strain as *L. rhamnosus*, one of the most frequently encountered species, along with *Lactobacillus casei*, in infections due to *Lactobacillus* (6). To our knowledge, this is the first reported case of recurrent bacteremia due to *L. rhamnosus* without endocarditis and the second case of *Lactobacillus*-associated meningitis (5, 6). Indeed, meningitis remains scarcely reported; Cannon et al., reviewing 241 cases of *Lactobacillus* infections, reported a unique case of meningitis (6). The sole published case concerned neonatal meningitis (5).

In the case reported here, infection was not associated with probiotic use or a colonic anomaly, which could have favored *Lactobacillus* translocation. However, *L. rhamnosus* is found in a wide range of dairy products and can be present in the intestinal flora (3, 18). The empirical use of vancomycin and ceftazidime could have favored the emergence and persistence of this opportunistic pathogen, which is naturally resistant to these antibiotics. Different studies have observed that *L. rh-*

amnosus is able to persist in the intestinal mucosa, and this could have favored the recurrence of the infection (1, 10).

Of the different molecules with proven action against *Lactobacillus*, erythromycin and clindamycin seem to be the most active (6). In our patient, clindamycin was essential to treatment since each time it was discontinued the infection recurred.

This case confirms the risk of *Lactobacillus* infection in immunocompromised patients, even without probiotic use, and the importance of highly active antibiotics such as clindamycin in limiting recurrence of the infection.

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